PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 15 February 2001 (15.02.01)	
International application No. PCT/CA00/00718	**Applicant's or agent's file reference** 10189- WJG
International filing date (day/month/year) 15 June 2000 (15.06.00)	**Priority date** (day/month/year) 18 June 1999 (18.06.99)
Applicant GAWAD, Yahia	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

09 January 2001 (09.01.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

A. Karkachi

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
10189- WJG		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/CA 00/ 00718	15/06/2000	18/06/1999
Applicant		
CARDIOGENICS, INC. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 2

   [X] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PC[redacted] 00/00718

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/533 G01N33/58

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, FSTA, INSPEC, COMPENDEX, BIOSIS, CHEM ABS Data, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 30908 A (DADE BEHRING INC) 16 July 1998 (1998-07-16) page 3, line 30 -page 4, line 23 page 6, line 26 -page 9, line 21 examples 1,2 ---	1-8,10, 14-19, 21-24, 26,33-40
X	EP 0 437 013 A (ELA TECHNOLOGIES INC) 17 July 1991 (1991-07-17) column 1, line 29 - line 56 column 4, line 12 - line 47 column 7, line 32 - line 58 claims 1-7 --- -/--	1,4-8, 10,14-19

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 October 2000	19/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Menidjel, R

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PC[redacted]A 00/00718

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	KENDALL J M ET AL: "Aequorea victoria bioluminescence moves into an exciting new era" TRENDS IN BIOTECHNOLOGY,GB,ELSEVIER PUBLICATIONS, CAMBRIDGE, vol. 16, no. 5, 1 May 1998 (1998-05-01), pages 216-224, XP004117786 ISSN: 0167-7799 page 216, right-hand column, paragraph 4 -page 217, right-hand column, paragraph 2 ---	1,3-8, 10,11, 13-19, 33-40
A	US 5 486 455 A (STULTS NANCY L) 23 January 1996 (1996-01-23) column 2, line 41 - line 64 column 4, line 1 - line 44 ---	21-32
P,X	WO 99 38999 A (PACKARD INSTRUMENT CO INC) 5 August 1999 (1999-08-05) abstract page 3, line 16 -page 5, line 2 -----	1,3,4, 6-8, 10-16, 18,19

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 00/00718

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9830908	A	16-07-1998	US	5876935 A	02-03-1999
			AU	5816798 A	03-08-1998
			BR	9805892 A	24-08-1999
			CN	1216112 A	05-05-1999
			CN	1216112 T	05-05-1999
			EP	0956507 A	17-11-1999
			JP	2000508075 T	27-06-2000
EP 0437013	A	17-07-1991	CN	1046605 A	31-10-1990
			JP	3067156 A	22-03-1991
			NO	901613 A	11-10-1990
			ZA	9002711 A	24-12-1991
US 5486455	A	23-01-1996	US	5648218 A	15-07-1997
			AU	6171894 A	29-08-1994
			EP	0683822 A	29-11-1995
			IL	108607 A	06-12-1998
			JP	8506897 T	23-07-1996
			MX	9401112 A	31-08-1994
			WO	9418342 A	18-08-1994
WO 9938999	A	05-08-1999	AU	2323399 A	16-08-1999

PCT REQUEST

10189- WJG

Original (for **SUBMISSION**) - printed on 15.06.2000 02:35:13 PM

0 0-1	**For receiving Office use only** International Application No.	
0-2	International Filing Date	
0-3	Name of receiving Office and "PCT International Application"	

0-4 0-4-1	**Form - PCT/RO/101 PCT Request** Prepared using	PCT-EASY Version 2.90 (updated 10.05.2000)
0-5	**Petition** The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	**Receiving Office (specified by the applicant)**	Canadian Patent Office (RO/CA)
0-7	**Applicant's or agent's file reference**	10189-5 WJG
I	**Title of invention**	METHOD FOR CONDUCTING CHEMILUMINESCENT BINDING ASSAY
II II-1 II-2 II-4 II-5 II-6 II-7 II-8 II-9	**Applicant** This person is: Applicant for Name Address: State of nationality State of residence Telephone No. Facsimile No.	 applicant only all designated States except US CARDIOGENICS, INC. 208 Evans Avenue Suite 214 Toronto, Ontario M8Z 1J7 Canada CA CA 416-251-2890 416-251-5133
III-1 III-1-1 III-1-2 III-1-4 III-1-5 III-1-6 III-1-7	**Applicant and/or inventor** This person is: Applicant for Name (LAST, First) Address: State of nationality State of residence	 applicant and inventor US only GAWAD, Yahia 2121 Rathburn Avenue Apartment 1110 Mississauga, Ontario L4W 2X3 Canada CA CA

PCT REQUEST 10189-WJG

Original (for **SUBMISSION**) - printed on 15.06.2000 02:35:13 PM

IV-1	**Agent or common representative; or address for correspondence** The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:	agent
IV-1-1	Name (LAST, First)	GALLOWAY, Warren, John
IV-1-2	Address:	Sim & McBurney 330 University Avenue Sixth Floor Toronto, Ontario M5G 1R7 Canada
IV-1-3	Telephone No.	416-595-1155
IV-1-4	Facsimile No.	416-595-1163
IV-1-5	e-mail	galloway@sim-mcburney.com
V	**Designation of States**	
V-1	Regional Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AP: GH GM KE LS MW MZ SD SL SZ TZ UG ZW and any other State which is a Contracting State of the Harare Protocol and of the PCT EA: AM AZ BY KG KZ MD RU TJ TM and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT EP: AT BE CH&LI CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE and any other State which is a Contracting State of the European Patent Convention and of the PCT OA: BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG and any other State which is a member State of OAPI and a Contracting State of the PCT
V-2	National Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AE AG AL AM AT AU AZ BA BB BG BR BY CA CH&LI CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

PCT REQUEST 10189- WJG

Original (for **SUBMISSION**) - printed on 15.06.2000 02:35:13 PM

V-5	**Precautionary Designation Statement** In addition to the designations made under items V-1, V-2 and V-3, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except any designation(s) of the State(s) indicated under item V-6 below. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit.		
V-6	**Exclusion(s) from precautionary designations**	NONE	
VI-1	**Priority claim of earlier national application**		
VI-1-1	Filing date	18 June 1999 (18.06.1999)	
VI-1-2	Number	60/139,941	
VI-1-3	Country	US	
VII-1	**International Searching Authority Chosen**	European Patent Office (EPO) (ISA/EP)	
VIII	**Check list**	number of sheets	electronic file(s) attached
VIII-1	Request	4	-
VIII-2	Description	20	-
VIII-3	Claims	8	-
VIII-4	Abstract	1	abstract.txt
VIII-5	Drawings	7	-
VIII-7	TOTAL	40	
	Accompanying items	paper document(s) attached	electronic file(s) attached
VIII-8	Fee calculation sheet	✓	-
VIII-16	PCT-EASY diskette	-	diskette
VIII-18	**Figure of the drawings which should accompany the abstract**	2	
VIII-19	**Language of filing of the international application**	English	
IX-1	**Signature of applicant or agent**		
IX-1-1	Name (LAST, First)	GALLOWAY, Warren, John	

FOR RECEIVING OFFICE USE ONLY

10-1	**Date of actual receipt of the purported international application**	
10-2	**Drawings:**	
10-2-1	Received	
10-2-2	Not received	

PCT REQUEST 10189- WJG

Original (for **SUBMISSION**) - printed on 15.06.2000 02:35:13 PM

10-3	Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application	
10-4	Date of timely receipt of the required corrections under PCT Article 11(2)	
10-5	International Searching Authority	ISA/EP
10-6	Transmittal of search copy delayed until search fee is paid	

FOR INTERNATIONAL BUREAU USE ONLY

11-1	Date of receipt of the record copy by the International Bureau	

PCT (ANNEX - FEE CALCULATION SHEET)

10189- WJG

Original (for **SUBMISSION**) - printed on 15.06.2000 02:35:13 PM

(This sheet is not part of and does not count as a sheet of the international application)

0 0-1	**For receiving Office use only** International Application No.				
0-2	Date stamp of the receiving Office				
0-4 0-4-1	**Form - PCT/RO/101 (Annex)** **PCT Fee Calculation Sheet** Prepared using	PCT-EASY Version 2.90 (updated 10.05.2000)			
0-9	**Applicant's or agent's file reference**	10189- WJG			
2	**Applicant**	CARDIOGENICS, INC., et al.			
12	**Calculation of prescribed fees**	fee amount/multiplier		total amounts (CAD)	
12-1	Transmittal fee T	⇨		200	
12-2	Search fee S	⇨		1,353	
12-3	International fee Basic fee (first 30 sheets) b1	630			
12-4	Remaining sheets	10			
12-5	Additional amount (X)	15			
12-6	Total additional amount b2	150			
12-7	b1 + b2 = B	780			
12-8	Designation fees Number of designations contained in international application	86			
12-9	Number of designation fees payable (maximum 8)	8			
12-10	Amount of designation fee (X)	136			
12-11	Total designation fees D	1,088			
12-12	PCT-EASY fee reduction R	-194			
12-13	Total International fee (B+D-R) I	⇨		1,674	
12-17	**TOTAL FEES PAYABLE (T+S+I+P)**	⇨		3,227	
12-19	**Mode of payment**	cheque			
12-20	**Deposit account instructions** The receiving Office:	Canadian Patent Office (RO/CA)			
12-20-2	is hereby authorized to charge any deficiency or credit any over-payment in the total fees indicated above to my deposit account	✓			
12-20-3	is hereby authorized to charge the fee for preparation and transmittal of the priority document to the International Bureau of WIPO to my deposit account	✓			
12-21	Deposit account No.	00000			
12-22	Date	15 June 2000 (15.06.2000)			

PCT (ANNEX - FEE CALCULATION SHEET) 10189- WJG

Original (for **SUBMISSION**) - printed on 15.06.2000 02:35:13 PM

12-23	Name and signature	GALLOWAY, Warren, John

VALIDATION LOG AND REMARKS

13-2-6	Validation messages Contents	Yellow! The power of attorney or a copy of the general power of attorney will need to be furnished unless all applicants sign the request form.
		Green? Priority 1. The priority document is not enclosed. (The applicant must furnish it within 16 months from the earliest priority date claimed)

PCT

10189- WJG

Original (for **SUBMISSION**) - printed on 15.06.2000 02:35:13 PM

PCT-EASY INFORMATION SHEET

(For applicant use only, DO NOT submit this sheet with the international application)

VALIDATION LOG

	Contents
Yellow!	The power of attorney or a copy of the general power of attorney will need to be furnished unless all applicants sign the request form.
Green?	Priority 1. The priority document is not enclosed. (The applicant must furnish it within 16 months from the earliest priority date claimed)

Before submitting the International Application, please carefully verify that:

-the information contained on printed Request form is correct;
-Box IX of the Request form has been signed;
-all elements of the international application as indicated in Box VIII of the Request form have been attached; and,
-the diskette containing the PCT-EASY zip file of the International Application has been enclosed and has been clearly labeled "PCT-EASY", with the applicant's or agent's file reference, and the first applicant's name.

ATTENTION

DO NOT modify any indications on the Request form printout. The attached PCT-EASY application has been locked. If an error or an omission is discovered at this time, you must copy the submitted application as a template and make the change or correction in a new application (using the submitted application as a template). You may create such a template by copying the submitted application from the "Stored Forms" folder to the "New PCT Forms" folder. Open the new (.0WO) file created in the "New PCT Forms" folder, correct the errors and proceed with the submission process again.

The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are with the one chosen by the applic[redacted] The full name or two-letter code of that Autho[redacted]y be indicated by the applicant on the line

IPEA/ _____

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only

Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION

Applicant's or agent's file reference: 10189-5 WJG

International application No.	International filing date *(day/month/year)*	(Earliest) Priority date *(day/month/year)*
PCT/CA00/00718	15 JUNE 2000 (15/06/2000)	18 JUNE 1999 (18/06/1999)

Title of invention
METHOD FOR CONDUCTING CHEMILUMINESCENT BINDING ASSAY

Box No. II APPLICANT(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

CARDIOGENICS, INC.
208 Evans Avenue
Suite 214
Toronto, Ontario
CANADA M8Z 1J7

Telephone No.: 416-251-2890
Facsimile No.: 416-251-5133
Teleprinter No.:

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
CA	CA

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

GAWAD, Yahia
2121 Rathburn Avenue
Apartment 1110
Missisauga, Ontario
CANADA L4W 2X3

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
CA	CA

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is, country)* of nationality:	State *(that is, country)* of residence:

☐ Further applicants are indicated on a continuation sheet.

International application No.
PCT/CA00/00718

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is ☒ agent ☐ common representative

and ☒ has been appointed earlier and represents the applicant(s) also for international preliminary examination.

☐ is hereby appointed and any earlier appointment of (an) agent(s) /common representative is hereby revoked.

☐ is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official The address must include postal code and name of country.)*

GALLOWAY, Warren J.
Sim & McBurney
330 University Avenue
Sixth Floor
Toronto, Ontario
M5G 1R7 CANADA

Telephone No.: 416-595-1155

Facsimile No.: 416-595-1163

Teleprinter No.:

☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

1. The applicant wishes the international preliminary examination **to start on the basis of:**

☒ the international application as originally filed.

the description ☒ as originally filed
☐ as amended under Article 34

the claims ☒ as originally filed
☐ as amended under Article 19 (together with any accompanying statement)
☐ as amended under Article 34

the drawings ☒ as originally filed
☐ as amended under Article 34

2. ☐ The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. ☐ The applicant wishes the start of the international preliminary examination **to be postponed** until the expiration of 20 months from the priority date unless the International Preliminary Examing Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: English

☒ which is the language in which the international application was filed.
☐ which is the language of a translation furnished for the purposes of international search.
☐ which is the language of publication of the international application.
☐ which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The applicant hereby **elects all eligible States** *(that is, all States which have been designated and which are bound by Chapter II of the PCT)*

excluding the following States which the applicant wishes **not to elect:**

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

GALLOWAY, Warren J
SIM & McBURNEY
330 University Avenue
Sixth Floor
Toronto, Ontario M5G 1R7
CANADA

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	15.02.2001
Applicant's or agent's file reference	IMPORTANT NOTIFICATION
10189-5 WJG	

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA00/00718	15/06/2000	18/06/1999

Applicant
CARDIOGENICS, INC. et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Danti, B Tel.+49 89 2399-8161

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
10189-5 WJG		

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA00/00718	15/06/2000	18/06/1999

International Patent Classification (IPC) or national classification and IPC
G01N33/533

Applicant
CARDIOGENICS, INC. et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
09/01/2001	15.02.2001

Name and mailing address of the international preliminary examining authority:
European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer
Thiele, U
Telephone No. +49 89 2399 8643

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA00/00718

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office i response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-20 as originally filed

Claims, No.:

1-40 as originally filed

Drawings, sheets:

1/7-7/7 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-40
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-40
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-40
	No:	Claims	

2. Citations and explanations
see separate sheet

S ction V

1) Reference is made to the following documents:

D1: WO 98 30908 A (DADE BEHRING INC) 16 July 1998 (1998-07-16)
D2: EP-A-0 437 013 (ELA TECHNOLOGIES INC) 17 July 1991 (1991-07-17)
D3: US-A-5 486 455 (STULTS NANCY L) 23 January 1996 (1996-01-23)
D4: WO 99 38999 A (PACKARD INSTRUMENT CO INC) 5 August 1999 (1999-08-05)

2) The subject-matter of claim 1 would appear to be novel and inventive in view of the known state of the art (Art. 33(2), (3) PCT).

D1 (see page 3, line 30 - page 4, line 23; page 6, line 26 - page 9, line 21) merely discloses a specific binding assay utilizing aequorin as the luminescent label. To this end and in one possible format, the luminescent label is conjugated to a first binding reagent capable of binding to the analyte. The second binding reagent is a biotinylated monoclonal antibody that is capable of binding to the analyte. Also provided is a solid support consisting of streptavidin-coated magnetic particles. Upon formation of the immunocomplex and binding to the magnetic particles, sufficient calcium ions are added to cause the emission of light by the luminescent aequorin label.

D2 (col. 1, lines 29 - 56; col. 4, lines 12 - 47; col. 7, lines 32 - 58) relates to a method for increasing sensitivity of luminescence assays for analytes. Luminescence, by e.g. an aequorin label, is measured after adding an appropriate trigger, e.g. calcium to the medium being measured in order to induce luminescence to occur.

D3 (see col. 7, lines 1 - 21) discloses nothing relevant which would go beyond the teaching of D1 and D3. The same applies to D4, which is cited "P,X" in the international search report, and which is considered to represent distant state of the art.

Calcium caging compounds would appear to exist in the art, i.a. as photosensitive

derivatives of chelators with known high affinity for calcium (see present description, page 5.

The skilled person, being equipped with the known state of the art discussed above, would however have had neither motivation nor guidance to adapt the assays of D1 and D2 to a test strip format where on the test strip a calcium-caging compound is contained in a transverse stripe, the immunocomplexes are mobilized on the strip in a predetermined direction along one side of an elongated matrix so as to contact the said stripe, and where calcium is released from the calcium caging compound upon exposure to a pulse of ultraviolet light so as to trigger emission of luminescence.

The advantages of the present invention are apparent from the description, page 18, lines 10 et seq.

3) The methods of claims 21 and 23 rely on the same essential technical features as the subject-matter of claim 1.

Thus, the presence of novelty and inventiveness can for analogous reasons be acknowledged for the subject-matter of claims 21 and 23, and for dependent claims 2 - 20, 22 and 24 - 27 (Art. 33(2), (3) PCT).

4) The devices of claims 28 and 33 would appear to be novel (Art. 33(2) PCT) already for the presence of the feature "capture strip having a transverse section thereof impregnated with streptavidin and a calcium caging compound".

Moreover, the apparatus of claim 37 comprising i.a. a means for removing a light protective barrier over the transverse stripe, an UV light source and a photomultiplier would appear to comply with the requirements for novelty (Art. 33(2) PCT).

5) Since finally the devices of claims 28, 33 and 37 are specially adapted to the novel and inventive claimed methods, for the said devices also the presence of inventive step can be acknowledged (Art. 33(3) PCT).

6) Claims 29 - 32, 34 - 36 and 38 - 40 comply with Arts. 33(2) and (3) PCT by virtue of their dependence.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 December 2000 (28.12.2000)

PCT

(10) International Publication Number
WO 00/79276 A1

(51) International Patent Classification[7]: G01N 33/533, 33/58

(21) International Application Number: PCT/CA00/00718

(22) International Filing Date: 15 June 2000 (15.06.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/139,941 18 June 1999 (18.06.1999) US

(71) Applicant *(for all designated States except US)*: CARDIOGENICS, INC. [CA/CA]; Suite 214, 208 Evans Avenue, Toronto, Ontario M8Z 1J7 (CA).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: GAWAD, Yahia [CA/CA]; Apartment 1110, 2121 Rathburn Avenue, Mississauga, Ontario L4W 2X3 (CA).

(74) Agent: GALLOWAY, Warren, John; Sim & McBurney, Sixth Floor, 330 University Avenue, Toronto, Ontario M5G 1R7 (CA).

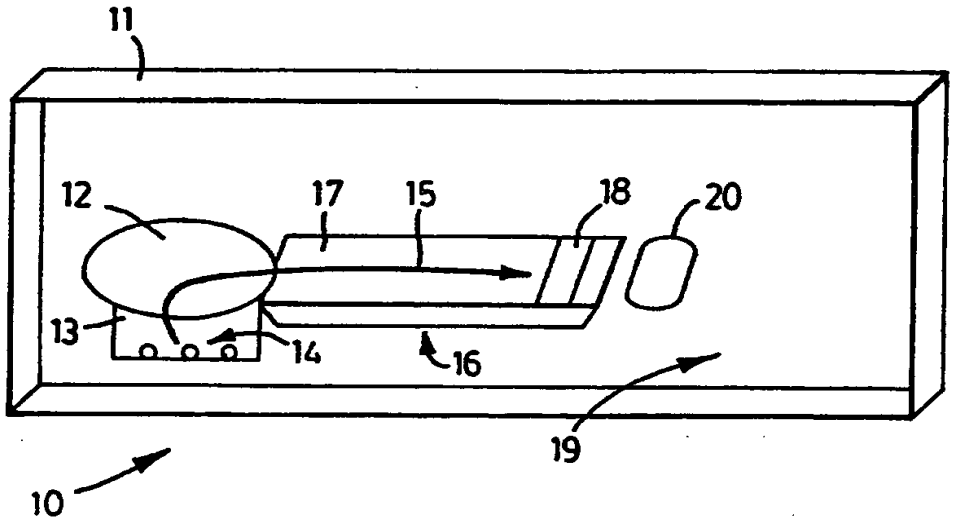
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

[Continued on next page]

(54) Title: METHOD FOR CONDUCTING CHEMILUMINESCENT BINDING ASSAY



(57) Abstract: A method for conducting a receptor-ligand binding reaction of a solution containing or suspected of containing the target analyte. The method comprises the steps of bonding the first binding partner to the surface of a paramagnetic particle, conjugating a second binding partner to a calcium-sensitive luminescent compound; contacting the first and second binding partners with the solution to be tested, immobilizing the paramagnetic particles along a capture strip that has a transverse stripe containing streptavidin and containing a caged calcium compound, exposing the transverse stripe to a pulse of ultraviolet light to effect the release of calcium from the caged calcium compound, and measuring luminescence emitted by the calcium-sensitive luminescent material. The method may be used in the testing of blood. An apparatus is also disclosed.

WO 00/79276 A1

— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

TITLE:
METHOD FOR CONDUCTING CHEMILUMINESCENT BINDING ASSAY

Field of the Invention

The present invention relates to a method for conducting a binding assay, and in particular to an immunoassay method that may be conducted on a Point Of Care (POC) device or an autoanalyzer.

Background to the Invention

The on-going needs to detect and quantify biomolecules (analytes) in various body fluids have resulted in the introduction of new and more accurate analytical techniques that can be adapted for measuring a wide spectrum of different analytes. Most of these detection methods have been introduced into the clinical diagnostic field in recent years. Currently, a broad expansion in both the variety of analytes that may be readily and accurately determined as well as the methods for the determination have been witnessed. However, convenient, reliable, non-hazardous, highly sensitive and technically less challenging methods for detecting the presence of low concentrations of analytes in liquids are still desired, especially when the analyte may be present in body fluids in very low concentrations.

Several methods for the detection and quantification of substances of biological origin in fluid samples are currently employed. Bioanalytical assays, such as immunoassays and nucleic acid hybridization assays, which are based on the specific binding between ligands and one or more members of specific binding pairs are widely used to determine the presence and quantity of analytes of interest, for example chemical constituents or substances of a sample. In particular, immunoassays are widely employed detection and quantification methods in the clinical laboratory.

In a typical procedure of a sandwich immunoassay, an antibody against a particular antigen, known as a capture antibody, is immobilized to a solid surface. The sample under investigation is contacted with the solid

surface under conditions that allow antigen in the sample to bind to the capture antibody. Another antibody known as a detector antibody is added. In the direct immunoassay format, the detector antibody is directly conjugated with a signal generating mechanism that allows the amount of the detector antibody to be quantified. In the indirect format, after the binding of the detector antibody to the antigen, another antibody against the detector antibody or another specific binding reaction that involves the detector antibody is utilized. This so-called anti-detector antibody is directly conjugated with a signal generating mechanism. The binding reaction and therefore the antigen level in the sample is quantified by quantifying the signal produced by the signal generating mechanism.

Several types of labeling material have been utilized for signal generation in the receptor-ligand binding assays. Radioactive atoms, such as ^{125}I, ^{131}I, ^{3}H and ^{14}C were commonly utilized as the label. Although radioactive labels for immunoassays are sensitive, they suffer commonly recognized disadvantages, including safety and the stringent regulatory requirements resulting in a relatively short reagent shelf life. Several alternative labeling methods are currently utilized in binding bioassays including colorimetric enzyme reactions, fluorescence and chemiluminescence reactions. Enzymes commonly utilized as labels are horseradish peroxidase, alkaline phosphatase, B-galactosidase and glucose oxidase. Although enzymes have an advantage over radioactive labels in that they are very stable and need no special facilities and instrumentation, enzyme immunoassays are generally slower, laborious and less sensitive. Luminescent labels, including fluorescent and chemiluminescent labels, have been utilized as an alternative for radioactive or enzyme labels as they possess the ease of use advantage of radiolabels and the reagent stability advantage of enzymes. Fluorescence detection can be used with a much wider variety of enzymes. However, due to the difficulty of conventional fluorescence detection in discriminating between specific and nonspecific signals and therefore the practical assay detection limit, fluorescence assays lack the sensitivity of either radioactive or enzyme

labels, making them seldom the assay method of choice for both research and clinical applications.

Chemiluminescent reactions as label of signal generation are the most sensitive and have been around for decades. Recent advances in DNA technologies have expanded the utilization of these labels as signal generators, but due to the limited number of known reactions that form chemiluminescent products, the luminescence assay method is currently under utilized. Also, luminescent reactions need one or more chemical activation steps, and automation of these reactions is difficult, although needing less complex instrumentation than fluorescence. Even though a large number of luminescence meters viz. luminometers, of various formats and sizes are available, automation of luminescence is complicated and fully-automated luminometers for carrying out binding assays are not available, at least in convenient, small-size analyzers.

The most common luminescence method utilized as a label for signal generation in binding assays is chemiluminescence. This may be classified according to the method utilized for generating the luminescent signal viz. chemiluminescent and bioluminescent labels. Bioluminescence refers to the emission of light by biological molecules and utilizes bioluminescent proteins which can be true enzymes. Examples are luciferases that catalyze the oxidation of luciferin with release of oxyluciferin and emit light, and photoproteins that catalyze the oxidation of luciferin to emit light but do not release the oxidized substrate.

The calcium-sensitive photoproteins, including Aequorin, Obeln, Mnemiopsin, Berovin, Pholasin, Luciferases and photoproteins isolated from Pelagia, Cypridina and ostracods were widely researched and employed in binding assays. Furthermore, the genes of some of them have been cloned, permitting the production of large quantities. Aequorin is the most commonly studied and employed member of this group of calcium-sensitive photoproteins.

Native aequorin, isolated from jellyfish (Aequorea), has been purified and utilized as a label in varieties of monitoring systems. Native aequorin

consists of a single polypeptide chain of MW 21,000 Daltons (called apoaequorin), containing one mole each of tightly bound coelenterate luciferin and oxygen. This complex is stable in the absence of calcium ions. Aequorin can also be produced by recombinant DNA techniques, for example as discussed by Cormier, M. J., U.S. Patent 5,162,227 and Zenno. S. et al. in U.S. Patent 5,288,623. Furthermore, modified forms of aequorin with enhanced bioluminescence properties have also been produced by recombinant DNA procedures, as disclosed by Prasher, D. in U.S. Patent 5,360,728.

The mechanism of photon emission of aequorin is well understood. Aequorin has a high-affinity for calcium ions. In the presence of excess calcium ions, aequorin catalyzes the oxidation of luciferin to oxyluciferin in a single turnover event with the generation of a glow-type "flash reaction" which persists for approximately 10 seconds with a relatively high quantum yield. Although peak light emission is initiated upon binding of three moles of calcium ions per mole of aequorin, binding of aequorin with trace of amount of free calcium results in partial oxidation of coelenterazine and yields apoaequorin, coelenteramide, CO and light.

As aequorin can be detected at the attomole level and the wavelength of its luminescence is very narrow and may be detected using commercially available luminometers, luminescence of aequorin offers many advantages including speed, high sensitivity and accuracy with a low background. Therefore, aequorin has proven useful as a label in binding assays. Furthermore, stable conjugates of aequorin with various binding reagents such as receptors, hormones, lectins, antibodies, antigens, DNA, RNA, oligonucleotides, and glycoproteins have been developed and a large number of such conjugates are commercially available.

When utilized in combination with streptavidin, biotinylated derivative of aequorin demonstrates the ability to detect nanogram to subnanogram amounts of the target analyte, including proteins and DNA, immobilized onto the wells of microtiter plates or nitrocellulose membranes. Marketed luminometers that employ aequorin are designed with injectors to inject

calcium at a particular moment. Although several clinical testing assays that utilize aequorin have been introduced, the luminometers are not automated and tanks of solutions of calcium have to be included. This makes them awkward to use by non-specialized personnel. A luminescent binding assay that utilizes aequorin and whole blood is disclosed by Pankratz et al in U.S. Patent No. 5,876,935.

In the cell of an organism, calcium (Ca) is an important intracellular second messenger for a wide variety of processes, which have physiological, biochemical and pathophysiological significance such as muscle contraction, neurotransmitter release, ion channel gating and exocytosis. Attempts to understand and measure the rapid changes and release of intracellular calcium have resulted into the introduction of a class of calcium-sensitive compounds called calcium-caging compounds. Calcium-caging compounds have the ability to be loaded with calcium and to unload their calcium upon stimulation. Unloading of the encased calcium may be induced by several methods, one of which is through exposure to light. Light-stimulation release of calcium from the caged compounds (called photolysis) is usually done by illumination for fractions of a second with laser pulses typically in the UV 350-400 nm region of the spectrum. Two different classes of Ca-caging compounds have been introduced; the BAPTA derivative such as the nitr-5 and nitr-7 and the EDTA or EGTA derivatives such as DM-nitrophen and nitrophenyl-EGTA. BAPTA is 1,2-bis(ortho-aminophenoxy) ethane-N,N,N',N'-tetraacetic acid. Nitr-7 is cis-1-(2-bis(carboxymethyl)amino-5-(1-hydroxy-1-(2-nitro-4,5-methylenedioxyphenyl)methyl)phenoxy)-2-(2-bis(carboxymethyl)amino-5-methylphenoxy)cyclopentane. Nitr-5 is 1-[2-Amino-5-(1-hydroxy-1-[2-nitro-4,5-methylenedioxyphenyl]methyl)phenoxy]-2-)2'-amino-5'methylphenoxy)ethane-N,N,N',N'-tetraacetic acid. DM-nitrophen is 1-(4,5 dimethoxy-2-nitrophenyl)-1,2 diaminoethane-N, N, N', N'-tetraacetic acid and nitrophenyl-EGTA i.e. nitrophenyl ethylenebis(oxyethylenenitrilo) tetraacetic acid. The latter class was designed to produce photosensitive derivatives of chelators with known high affinity for calcium, see US Patent 5,446,186 and U.S. Patent 4,981,985. The DM-nitrophen and nitrophenyl-

EGTA calcium-caging compounds offer the advantage of calcium-selectivity. On irradiation, the chelated calcium cleaves with the subsequent cleaved remainders having a substantially lower affinity for the released calcium. Thus, large mounts of calcium are rapidly released. These photosensitive calcium-caging compounds are commercially available.

A binding assay e.g. immunoassay or nucleic acid binding assay, that utilizes photosensitive calcium-caging compounds would be useful.

Summary of the Invention

A method has now been found for conducting a receptor-ligand binding assay utilizing calcium caging compounds and calcium-sensitive luminescent compounds.

Accordingly, one aspect of the present invention provides a method for conducting a binding assay to detect the presence of an analyte in a solution, comprising the steps of:

(a) contacting a first binding partner with said solution, said first binding partner being conjugated to a calcium-sensitive chemiluminescent material;

(b) after a period of time, mobilizing the first binding partner in a predetermined direction along one side of an elongated matrix of a capture strip so as to contact the first binding partner with a stripe transversely located on said capture strip, said transverse stripe having immobilized second binding partner and containing a calcium-caging compound,

(c) allowing a period of time sufficient for the first binding partner to contact said second binding partner immobilized onto said transverse stripe,

(d) exposing said transverse stripe of said capture strip to a pulse of ultraviolet light to effect the release of calcium from the calcium caging compound; and

(e) measuring luminescence emitted by the calcium-sensitive luminescent material.

Another aspect of the present invention provides a method for conducting a binding assay to detect the presence of an analyte in a solution, comprising the steps of:

(a) contacting said solution with a first binding partner of a binding reaction, said first binding partner being immobilized on a solid surface, said solid surface being paramagnetic particles and said first binding partner being conjugated to calcium-sensitive luminescent material;

(b) after a period of time, mobilizing the paramagnetic particles in a predetermined direction along one side of an elongated matrix of a capture strip so as to contact the particles with a stripe of a second binding partner transversely located on said capture strip, said capture strip having the second binding partner immobilized onto said transverse stripe, said transverse stripe additionally containing a calcium-caging compound,

(c) allowing a period of time sufficient for the paramagnetic particles to contact said second binding partner immobilized onto said transverse stripe,

(d) exposing said transverse stripe of said capture strip to a pulse of ultraviolet light to effect the release of calcium from the calcium caging compound; and

(e) measuring luminescence emitted by the calcium-sensitive luminescent material.

In preferred embodiments of the invention, the method is an immunoassay for detecting and quantifying an antigen, an immunoassay for detecting and quantifying an antibody, or a nucleic acid hybridization assay for detection and quantifying a particular sequence of nucleic acid.

In another embodiment, the solution is pretreated prior to contacting the calcium sensitive chemiluminescent material in step (a), especially filtered to remove calcium, the filter containing an agent for removal of calcium.

In another embodiment, the solution is whole blood, said whole blood being pretreated by filtering prior to being contacted with the paramagnetic particles.

In a further embodiment, the luminescent material is calcium-sensitive luminescent material, especially aequorin, Obeln, Mnemiopsin, Berovin, Pholasin, Luciferases or photoproteins isolated from Pelagia, Cypridina and ostracods.

In still further embodiments, the ultraviolet light is in the form of a pulse of light in the range of 250-400 nm, and the luminescence is measured by a photomultiplier. In particular, the calcium-sensitive luminescent material is aequorin and photomultiplier detects light of 400-600 nm and is protected from the magnetic field.

In other embodiments, the elongated capture strip is formed of nitrocellulose, polyacrylamide or other natural or synthetic polymer and has a transverse stripe with immobilized second binding partner and impregnated with a calcium caging compound.

In a further embodiment, the calcium-caging compound is loaded with calcium in excess of the stoichiometric amount for said calcium-sensitive luminescent material. Preferably, the calcium-caging compound is nitr-5, nitr-7, DM-nitrophen or nitrophenyl-EGTA.

A further aspect of the invention provides a method for conducting a binding assay to detect the presence of an analyte in a solution, comprising the steps of:

(a) immobilizing a first binding partner of a binding reaction onto a solid surface, said solid surface being paramagnetic particles, said first binding partner being biotinylated;

(b) contacting said first binding partner with said solution;

(c) contacting the solution with a second binding partner, said second binding partner being conjugated to a calcium-sensitive luminescent material;

(d) after a period of time, mobilizing the paramagnetic particles in a predetermined direction along one side of an elongated matrix of a capture strip so as to contact the particles with a stripe transversely located on said capture strip, said capture strip having streptavidin immobilized onto said

transverse stripe, said transverse stripe additionally contain a calcium-caging compound,

(e) allowing a period of time sufficient for the paramagnetic particles to contact said streptavidin immobilized onto said transverse stripe,

(f) exposing said transverse stripe of said capture strip to a pulse of ultraviolet light to effect the release of calcium from the calcium caging compound; and

(g) measuring luminescence emitted by the calcium-sensitive luminescent material.

In an embodiment, steps (b) and (c) are carried out simultaneously.

Yet another aspect of the invention provides a method for conducting a binding assay to detect the presence of an analyte in a solution, comprising the steps of:

(a) contacting a first binding partner with said solution, said first binding partner being biotinylated;

(b) after a period of time, contacting the solution with a second binding partner, said second binding partner being conjugated to a calcium-sensitive luminescent material;

(c) after a further period of time, mobilizing the binding partners in a predetermined direction along one side of an elongated matrix of a capture strip so as to contact the binding partners with a stripe transversely located on said capture strip, said capture strip having streptavidin immobilized onto said transverse stripe, said transverse stripe additionally contain a calcium-caging compound,

(d) allowing a period of time sufficient for the binding partners to contact said streptavidin immobilized onto said transverse stripe,

(e) exposing said transverse stripe of said capture strip to a pulse of ultraviolet light to effect the release of calcium from the calcium caging compound; and

(f) measuring luminescence emitted by the calcium-sensitive luminescent material.

In an embodiment, steps (a) and (b) are carried out simultaneously.

In a further embodiment, the elongated capture strip has a transverse stripe impregnated with streptavidin and a calcium-caging compound.

In a still further aspect, the present invention provides an elongated capture strip for binding assays, said strip having a transverse section thereof impregnated with a binding partner and a caged calcium compound.

In preferred embodiments, the capture strip is formed from nitrocellulose, polyacrylamide, polyamide or any other synthetic or naturally occurring polymer.

In another embodiment, the capture strip is in a housing, especially within a support as a single use testing cartridge.

In a further embodiment, the binding partner is streptavidin.

A further embodiment of the invention provides a plastic cartridge comprising:

a housing with a receptacle for receipt of a sample, a reservoir containing biotinylated first binding partner immobilized onto paramagnetic particles and a second binding partner conjugated to calcium-sensitive chemiluminescence material, an elongated capture strip within the housing and in fluid communication with the reservoir, said capture strip having a transverse section thereof impregnated with streptavidin and a calcium-caging compound, said transverse section being protected with a light barrier.

In a preferred embodiment, there is a filter between the receptacle and the reservoir, especially a filter containing an agent for removal of calcium.

A further embodiment provides apparatus for carrying out a binding assay comprising a housing enclosing (a) a receptacle to receive the said plastic cartridge; (b) a means for removing the light protective layer over the transverse stripe; (c) a electromagnet to provide a magnetic field; (e) a ultraviolet light source to project light on a pre-selected portion of the capture strip, and (f) a photomultiplier disposed to receive light emitted by the pre-selected portion of the capture strip.

Brief Description of the Drawings

The present invention is illustrated by the embodiment shown in the drawings, in which:

Fig. 1 is a schematic representation of a capture strip of the present invention;

Fig. 2 is a schematic representation of the cartridge of the present invention;

Fig. 3 is a schematic representation of apparatus of the present invention;

Fig. 4 is a graphical representation of photoemission from a sample in Example I;

Fig. 5 is a graphical representation of photoemission from a sample in Example I, after photolysis with ultraviolet light;

Fig. 6 is a graphical representation of the combined graphs of Fig.s 5 and 6;

Fig.s 7 and 8 are graphical representations of photoemission from samples in Example II.

Detailed Description of the Invention

While the present invention may be used for detection and quantification of a binding partner of a binding reaction, it will be described herein with particular reference to a sandwich immunoassay for the detection and quantification of antigen that additionally employs a biotin-streptavidin reaction and paramagnetic particles, which is preferred.

Fig. 1 shows a capture strip, generally indicated by 1. Capture strip 1 has an elongated matrix 2. Elongated matrix 2 is formed from a matrix composition that will permit the paramagnetic particles with associated immune complex thereon to pass along the capture strip under the influence of a magnetic field. Examples of the matrix composition include nitrocellulose, polyacrylamide, polyamide or other synthetic or naturally-occurring polymer. In other embodiments, the matrix is in the form of a microfluidic channel, especially a channel etched into the capture strip. In this embodiment, a

matrix composition would not be required. The capture strip must be formed of a clear material, especially in the location of the transverse stripe 3, to permit passage of light. Examples of such materials include acrylic polymers, polystyrene, acrylonitrile-butadiene-styrene (ABS), polycarbonate and other transparent polymers.

Elongated matrix 2 has transverse stripe 3 located towards one end, such end being opposed to inlet end 4. Transverse stripe 3 contains both streptavidin and calcium-loaded calcium caging compounds 5 or such other compounds as are disclosed herein.

Fig. 2 shows a plastic cartridge for carrying out the immunoassay reaction, generally indicated by 10. Plastic cartridge 10 has cartridge housing 11. Cartridge housing 11 has a sample receiving receptacle that contains a filter 12, a reservoir 13 for housing the paramagnetic particles 14 and the second binding partner thereon, and particle path 15. Particle path 15 is in fluid communication with the reservoir 13 and leads from reservoir 13 into capture strip 16, where particle path 15 extends along elongated path 17 of capture strip 16 to transverse stripe 18. Filter 12, reservoir 13 and capture strip 16 are all located within a holder 19 that forms part of plastic cartridge 10. It is to be understood that at least transverse stripe 18 would have a peelable protective light barrier thereon which would be removed before use, i.e. before exposure to light from the light source. Additionally, the elongated capture strip is in communication with a discharge reservoir 20 at the opposite end of the sample receiving receptacle for receiving reagents that pass from the transverse stripe 18.

Fig. 3 shows a testing platform apparatus, generally indicated by 30. Testing platform apparatus 30 has housing 31. Within the housing 31 are plastic cartridge 32, electromagnet 33, ultraviolet light source 34 and photomultiplier 35. Plastic cartridge 32 has been described previously, and could be accommodated within the receptacle of the housing 31 of the platform apparatus 30. Electromagnet 33 extends for the length of plastic cartridge 32. Electromagnet 33 is preferably comprised of sectional pre-determined magnetic fields that facilitate mobilization of paramagnetic beads

(particles) along the elongated path 17 into capture strip 16. Ultraviolet light source 34 is directed at plastic cartridge 32 and, in particular, at transverse stripe 18 of plastic cartridge 32, which has been described previously. Photomultiplier 35 is also directed at transverse stripe 18.

Testing platform apparatus 30 additionally has display 36, which would typically be an LCD display. Housing 31 would also contain appropriate controls and associated computer hardware and software to permit appropriate interpretation of the results obtained.

In use, a sample containing an antigen e.g. blood, is placed on filter 12. Liquid containing the target analyte passes through filter 12 into reservoir 13, where it contacts the paramagnetic particles which has the biotinylated first binding partner (capture partner) immobilized onto it and the second binding partner conjugated to a calcium-sensitive luminescent label (detector partner). In addition, it is understood that when the cartridge is designed to detect and quantify an antigen, the first and second binding partners are antibodies. On the other hand, when the cartridge is designed for detecting an antibody, the first binding partner is an antigen while the second binding partner is an antibody.

The plastic cartridge 10, 32 is then placed in the testing platform 30 if it is not already located within the platform. It is understood that at least transverse stripe 18 of capture strip 16 of plastic cartridge 10 would need to be protected from light. Such protection could be removed within testing platform 30, in a light-tight manner. Such removal could be automatic.

After allowing appropriate time for the binding reaction, the magnetic field is applied, using electromagnet 33. Then, the paramagnetic particles and attached immune complexes move along particle path 15 and into capture strip 16. The particles then pass along capture strip 16 until transverse stripe 18 is reached. At that time, the particles become bound to streptavidin, already located in transverse stripe 18, through the biotinylated binding partner immobilized onto the particles. Transverse strip 18 additionally contains a calcium caging compound.

After an appropriate time, which would depend in particular on the dimensions of the capture strip 16, but which conveniently could be 4-6 minutes, ultraviolet light source 34 is activated and sends a pulse of light onto transverse stripe 18. The light causes the release of calcium from the calcium-loaded calcium caging compound, which occurs essentially instantaneously. The calcium contacts the calcium-sensitive chemiluminescent material, which then glows for a short period of time in the range of 4-10 seconds. The light that is emitted is detected by the photomultiplier 35, and the amount of light emitted is interpreted and is displayed on display 36. The rate of emission of light depends on the energy of the ultraviolet light source. High energy levels will cause a high emission rate i.e. a sharp peak of emitted light, but it is preferred that lower energy levels be used such that the emitted light is a broader band. This will lead to more accurate recording of the amount of light by the photomultiplier, especially if emission of light commences prior to completion of the re-setting of the photomultiplier to its zero or null point, as discussed herein.

Some examples of the source of the solution containing or suspected of containing the target analyte that is subjected to the method of the present invention are blood or blood products, saliva, or any other body fluids. Other solutions could be tested.

Utilizing calcium-sensitive luminescent material as the signal generating label in binding assays requires that the solutions that will be contacting the calcium-sensitive luminescent conjugate have to be calcium-free before the moment of generating the light emission. Calcium in the solution will react with the calcium-sensitive luminescent conjugate. In particular, the solution should contain less than 20 nanomolar of calcium. Furthermore, when the goal is to determine the presence of an analyte in whole blood, the sample of blood normally must be pretreated to remove cellular components and hemoglobin, which can interfere with the specific signal of the binding assay. Filters impregnated in calcium-chelating agents would achieve both functions of removing the cellular components as well as calcium from the solutions that contain the target analyte.

The method of the invention disclosed herein utilizes any calcium-sensitive luminescent material for the signal generation in binding assays including, but not limited to, aequorin, mitrocomin, clytin, obelin, mnemiopsin, berovin, halistaurin and phialidin. In case of utilizing a calcium-sensitive luminescent photoprotein, other than aequorin, the optimal wavelength, other than 469 nm that is the optimal wavelength for detecting the aequorin signal, of the photomultiplier has to be adapted accordingly. For example, the wavelength may be 400-600 nm.

Photolysis of the calcium caging compounds may be achieved by many light sources generating light within a wavelength from 250-400 nm. Such light sources are referred to herein as ultraviolet light sources. One such source is a laser source, which is a convenient source to accurately deliver light for less than 1 millisecond at a wavelength of 300-350 nm. Upon the release of calcium from the caging compound when light-triggered and upon binding of three moles of calcium ions per mole of aequorin, the light emission is initiated with a flash of blue light that persists for approximately ten seconds. The generated light could then be measured with a suitable photomultiplier both as peak light or total photon counting.

The method of the present invention preferably utilizes a time-resolved mechanism, particularly time-resolved chemiluminescence. In this method, there is a short period of time between the flash emitted by the ultraviolet light source and the emission of light by the calcium-sensitive luminescent material. The calcium-sensitive luminescent material is selected to obtain such a period of time. The photomultiplier records the stray light after the flash from the ultraviolet source followed by a period of zero or substantially zero light, which is then followed by the emission of light. During the period of zero light, the reading on the photomultiplier can be re-set to its zero or null point, thereby permitting a more accurate reading of the emission of light. The period between the pulse of light and the emission of light is short, but such time is sufficient to reset the photomultiplier to a zero baseline.

The ultraviolet light source should be generally shielded from th capture strip, with the light being focussed on the transverse stripe, e.g. using

coated quartz lenses. As discussed herein, the intensity of the light source may be varied, but one embodiment is at least 150 mJ.

Native calcium-sensitive luminescent photoproteins are particularly useful as a label in the method of the present invention of carrying out binding assays. Other modified recombinant DNA-driven forms of these photoproteins with enhanced luminescence, due to either the ability of regeneration or a higher affinity for calcium, are also compatible with the method of the invention.

Although encasing compounds such as in light sensitive liposomes have been extensively researched, the recent introduction of cation-specific caging compounds is particularly useful in carrying out the method of the invention. The recently introduced two classes of calcium-caging compounds which are derivatives of chelating agents are particularly useful as they are more stable and the mechanism(s) of their triggering is well defined. In particular, the breakdown derivatives of DM-nitrophen derivative of EDTA (ethylenedinitrilo tetraacetic acid, disodium salt) and nitrophenyl-EGTA (ethylenebis(oxyethylenenitrilo) tetraacetic acid) have a very low affinity for calcium once light-triggered. Also, the wavelength of fluoroescence of the cleaved compounds is much different than that of the calcium-sensitive luminescent photoproteins and lasts for a very brief period of time. These photosensitive calcium-caging compounds are commercially available.

The detector materials on the transverse stripe may be located and immobilized on glass beads, which provides a high surface area of detector material.

Combining aequorin, which can be detected at the attomol level, together with exploiting the high affinity of biotin/streptavidin reaction offers a very high sensitivity of the method of the invention to measure analytes at a subnanogram level of detection. Furthermore, modified forms of streptavidin are also compatible with the method of the invention and both streptavidin and its derivatives could be easily immobilized onto the lateral transverse stripe of the capture matrix strip.

According to the method of the invention for carrying out a binding assay, separation of the bound from free luminescent label is effected by applying a magnetic field. It will be recognized that the force on suspended magnetic particle subjected to a magnetic field urges the particle to move to stronger field regions, typically towards the pole of a magnet, and that the strength of the force depends both on the field gradient and magnetism induced in the particle by the field. Thus, for rapid separation, a strong separator and a highly magnetizable particle appear preferable. Furthermore, the electromagnet is capable of producing several field gradients in pre-determined optimized directions.

Microscopic magnetic particles ranging from 0.7-1.5 microns are compatible with the method of the invention and may be used as they provide a large surface area for coating with proteins, for example, those disclosed in U.S. Patents No.s 3,970,518; 4,018,886; 4,230,685; 4,267,234; 4,452,773; 4,554,088; and 4,659,678. However, smaller size paramagnetic particles of the size 0.03 to 10, especially 0.5-1.0 micrometers, as described in the US Patent No. 5,736,349 are more suitable as large size particles of magnetic material tend to adhere to one another after removal of the magnetic field, due to residual magnetism. Suitable magnetic materials include ferromagnetic, ferrimagnetic and superparamagnetic materials. Other suitable magnetic materials include oxides, such as, for example, ferrites, perovskites, chromites and magnetoplumbites. Nickel particles may also be used.

The magnetic separation apparatus/method used for separating of target analyte-bearing magnetic particles from test media will depend on the nature and size of the magnetic particle. The micron-size magnetic particles suitable in the invention are readily removed from solution by means of commercially available magnetic separation devices. These devices employ a single relatively inexpensive permanent magnet located external to a container holding the test medium. Examples of such magnetic separators are the MAIA Magnetic Separator manufactured by Serono Diagnostics, Norwell, Mass., the DYNAL MPC-1 manufactured by DYNAL, Inc., Great Neck, N.Y. and the BioMag Separator, manufactured by Advanced Magnetics, Inc.,

Cambridge, Mass. The preferred magnetic separator for the present invention would have several aligned field gradients. In particular, multiple magnets could be used to effect stirring of the paramagnetic particles, and then to successively move the particles out of the vessel onto and along the capture strip. The magnets could be operated independently and/or in a coordinated sequence so as to effect stirring and then the movement of the particles along a pre-determined path e.g. to the capture strip and then to the transverse stripe. An example of the use of magnets in the stirring of magnetic particles is disclosed in US Patent No. 5,835,329.

In developing a bioassay, there are many considerations for the assay to attain value in the clinical laboratory. One consideration is the signal response to changes in the concentration of analyte. A second consideration is the ease with which the protocol for the assay may be carried out. A third consideration is the variation in interference from sample to sample. Also, ease of preparation and purification of the reagents, availability of equipment, ease of automation and interaction with material of interest are some of the additional considerations in developing a useful assay.

The method of the invention for carrying out a binding assay offers improvement in such consideration. The invention offers the high sensitivity of luminescence, the availability, sensitivity and high quantum yield of calcium-sensitive luminescent material, particularly aequorin, the physical characteristic of calcium-sensitive luminescent material to response to changes in calcium without having to manually inject calcium, the availability of commercial luminometers with photomultipliers that could detect the generated photons without the interference of the magnetic field, and the development of solid chromatographic capturing matrices that offer the convenience of point of care testing. Most important, the large difference in the wave length of exciting the caged calcium (240-400 nm) and the wavelength of measuring the generated photons (450-500 nm) facilitates detection of emitted light without interference from the incident light from the ultraviolet light source or due to fluorescence of the medium. Thus, a time-resolved chemiluminescence is used, as described herein.

The present invention of carrying receptor-ligand binding reaction utilizing a calcium-sensitive chemiluminescent label has been described herein with reference to the paramagnetic particle having the biotinylated first binding partner immobilized onto its surface, the chemiluminescent material conjugated to the second binding partner, and with the calcium caging compound being associated with the streptavidin to carry out a full sandwich immunoassay for detecting and quantifying an antigen as the preferred embodiment of the method invention. However, it is to be understood that the method of the invention is as equally beneficial in detecting an antibody as well as a nucleic acid as the target analyte of a receptor-ligand binding reaction. Also, it is to be understood that the method of the invention could be carried out with the first binding partner conjugated to a calcium-sensitive luminescent material and immobilized onto paramagnetic particles and the second binding partner immobilized in the transverse stripe of the capture strip together with the calcium caging compound.

The present invention is illustrated by the following examples.

EXAMPLE I

5 µg of aequorin in 10 µl was added to a 200 µl solution of buffered 1-(4,5 dimethoxy-2-nitrophenyl)-1,2 diaminoethane-N, N, N', N'-tetraacetic acid (DM-NP) containing calcium chloride. The solution contained 80 mM of 4-morpholine propane sulphonic acid (MOPS) buffer and 20 mM of KCl, with the pH of the solution adjusted to 7.2. The DM-NP was loaded with calcium up to 75% i.e. 2mM DM_NP + 1.5 mM $CaCl_2$.

Photoemission from the solution was monitored for 30 seconds at a wavelength of 470 nm. The results obtained shown in Fig. 4, shown minimal emission. The solution was then photolysed using an ultraviolet light of a wavelength of 347 nm. The pulse of light was 100 mJ.

The results obtained are shown in Fig. 5, and the results of Fig.s 4 and 5 are combined in Fig. 6.

The results show that the pulse of ultraviolet light caused release of sufficient calcium to trigger photoemission from aequorin. Prior to the pulse of

ultraviolet light (Fig. 4), the caged calcium did not trigger emission from aequorin. Emission of light was complete within 30 seconds.

EXAMPLE II

The procedure of Example I was repeated using solutions of 5 µg of aequorin. In separate experiments, 1mM of $CaCl_2$ and 500 µM of $CaCl_2$ were added. The total photon count after the pulse of ultraviolet light was monitored at 470 nm for 30 seconds.

The results are shown in Fig.s 7 and 8, respectively. Although the peak heights are different, the total amount of photons emitted is the same. Thus, the total amount of photons can be used to monitor the reaction. Under more controlled conditions i.e. not the manual addition of these examples, peak intensity could also be used.

CLAIMS:

1. A method for conducting a binding assay to detect the presence of an analyte in a solution, comprising the steps of:

(a) contacting a first binding partner with said solution, said first binding partner being conjugated to a calcium-sensitive chemiluminescent material;

(b) after a period of time, mobilizing the first binding partner in a predetermined direction along one side of an elongated matrix of a capture strip so as to contact the first binding partner with a stripe transversely located on said capture strip, said transverse stripe having immobilized second binding partner and containing a calcium-caging compound,

(c) allowing a period of time sufficient for the first binding partner to contact said second binding partner immobilized onto said transverse stripe,

(d) exposing said transverse stripe of said capture strip to a pulse of ultraviolet light to effect the release of calcium from the caged calcium compound; and

(e) measuring luminescence emitted by the calcium-sensitive luminescent material.

2. The method of Claim 1 for conducting a binding assay to detect the presence of an analyte in a solution, comprising the steps of:

(a) contacting said solution with a first binding partner of a binding reaction, said first binding partner being immobilized on a solid surface, said solid surface being paramagnetic particles and said first binding partner being conjugated to calcium-sensitive luminescent material;

(b) after a period of time, mobilizing the paramagnetic particles in a predetermined direction along one side of an elongated matrix of a capture strip so as to contact the particles with a stripe of a second binding partner transversely located on said capture strip, said capture strip having the second binding partner immobilized onto said transverse stripe, said transverse stripe additionally containing a calcium-caging compound,

(c) allowing a period of time sufficient for the paramagnetic particles to contact said second binding partner immobilized onto said transverse stripe,

(d) exposing said transverse stripe of said capture strip to a pulse of ultraviolet light to effect the release of calcium from the calcium caging compound; and

(e) measuring luminescence emitted by the calcium-sensitive luminescent material.

3. The method of Claim 2 in which the method is an immunoassay for detecting and quantifying an antigen, an immunoassay for detecting and quantifying an antibody, or a nucleic acid hybridization assay for detection and quantifying a particular sequence of nucleic acid.

4. The method of any one of Claims 1-3 in which the solution is pretreated prior to contacting the calcium sensitive luminescent material in step (a).

5. The method of Claim 4 in which the solution is filtered to remove calcium, the filter containing an agent for removal of calcium.

6. The method of any one of Claims 1-5 in which the solution is whole blood, said whole blood being pretreated by filtering prior to being contacted with the calcium sensitive luminescent material.

7. The method of any one of Claims 1-6 in which the calcium-sensitive luminescent material is aequorin, Obeln, Mnemiopsin, Berovin, Pholasin, Luciferases or photoproteins isolated from Pelagia, Cypridina and ostracods.

8. The method of any one of Claims 1-7 in which the ultraviolet light is in the form of a pulse of light in the range of 250-400 nm, and the luminescence is measured by a photomultiplier.

9. The method of Claim 8 in which the calcium-sensitive luminescent material is aequorin and in which the photomultiplier detects light of 400-600 nm and is protected from the magnetic field.

10. The method of any one of Claims 1-9 in which the elongated capture strip is formed of nitrocellulose, polyacrylamide or any other natural or synthetic polymer.

11. The method of Claim 10 in which the elongated capture strip has a transverse stripe with immobilized second binding partner and impregnated with a calcium caging compound.

12. The method of any one of Claims 1-11 in which the calcium caging compound is loaded with calcium in excess of the stoichiometric amount for said calcium-sensitive luminescent material.

13. The method of any one of Claims 1-12 in which the calcium-caging compound is selected from the group consisting of cis-1-(2-bis(carboxymethyl)amino-5-(1-hydroxy-1-(2-nitro-4,5-methylenedioxyphenyl)methyl)phenoxy)-2-(2-bis(carboxymethyl)amino-5-methylphenoxy)cyclopentane, 1-[2-Amino-5-(1-hydroxy-1-[2-nitro-4,5-methylenedioxyphenyl]methyl)phenoxy]-2-)2'-amino-5'methylphenoxy)ethane-N,N,N',N'-tetraacetic acid, 1-(4,5 dimethoxy-2-nitrophenyl)-1,2 diaminoethane-N, N, N', N'-tetraacetic acid and nitrophenyl-ethylenebis(oxyethylenenitrilo) tetraacetic acid.

14. The method of any one of Claims 1-13 which is an immunoassay for detecting and quantifying an antigen.

15. The method of any on of Claims 1-13 which is an immunoassay for detecting and quantifying an antibody.

16. The method of any one of Claims 1-13 in which the binding assay is nucleic acid hybridization assay for detection and quantifying a particular sequence of nucleic acid.

17. The method of any one of Claims 1-16 in which the calcium-sensitive luminescent material is aequorin.

18. The method of any one of Claims 1-17 in which the ultraviolet light source emits a pulse of light in the range of 250-400 nm.

19. The method of any one of Claims 1-18 in which the luminescence is measured by a photomultiplier.

20. The method of any one of Claims 1-19 in which the calcium-sensitive luminescent material is aequorin and photomultiplier detects light of 400-600 nm and is protected from the magnetic field.

21. A method for conducting a binding assay to detect the presence of an analyte in a solution, comprising the steps of:

(a) immobilizing a first binding partner of a binding reaction onto a solid surface, said solid surface being paramagnetic particles, said first binding partner being biotinylated;

(b) contacting said first binding partner with said solution;

(c) contacting the solution with a second binding partner, said second binding partner being conjugated to a calcium-sensitive luminescent material;

(d) after a period of time, mobilizing the paramagnetic particles in a predetermined direction along one side of an elongated matrix of a capture strip so as to contact the particles with a stripe transversely located on said capture strip, said capture strip having streptavidin immobilized onto said transverse stripe, said transverse stripe additionally contain a calcium-caging compound,

(e) allowing a period of time sufficient for the paramagnetic particles to contact said streptavidin immobilized onto said transverse stripe,

(f) exposing said transverse stripe of said capture strip to a pulse of ultraviolet light to effect the release of calcium from the calcium caging compound; and

(g) measuring luminescence emitted by the calcium-sensitive luminescent material.

22. The method of Claim 21 in which steps (b) and (c) are carried out simultaneously.

23. A method for conducting a binding assay to detect the presence of an analyte in a solution, comprising the steps of:

(a) contacting a first binding partner with said solution, said first binding partner being biotinylated;

(b) after a period of time, contacting the solution with a second binding partner, said second binding partner being conjugated to a calcium-sensitive luminescent material;

(c) after a further period of time, mobilizing the binding partners in a predetermined direction along one side of an elongated matrix of a capture strip so as to contact the binding partners with a stripe transversely located on said capture strip, said capture strip having streptavidin immobilized onto said transverse stripe, said transverse stripe additionally contain a calcium-caging compound,

(d) allowing a period of time sufficient for the binding partners to contact said streptavidin immobilized onto said transverse stripe,

(e) exposing said transverse stripe of said capture strip to a pulse of ultraviolet light to effect the release of calcium from the calcium caging compound; and

(f) measuring luminescence emitted by the calcium-sensitive luminescent material.

24. The method of Claim 23 in which steps (a) and (b) are carried out simultaneously.

25. The method of any one of Claims 21-24 in which the elongated capturing strip has a transverse section thereof impregnated with streptavidin and a calcium-caging compound.

26. The method of any one of Claims 1-25 in which the pulse of ultraviolet light and the detection of chemiluminescence are conducted in a time-resolved manner.

27. The method of any one of Claims 1-26 in which the solution contain less than 20 nanomolar of calcium before the pulse of ultraviolet light.

28. An elongated capture strip for binding assays, said strip having a transverse section thereof impregnated with streptavidin and a calcium caging compound.

29. The elongated capture strip of Claim 28 in which the capture strip is formed from nitrocellulose, polyacrylamide, polyamide or any other synthetic or naturally occurring polymer.

30. The elongated capture strip of Claim 28 or Claim 29 in which the capture strip is in a housing.

31. The elongated capture strip of Claim 30 in which the capture strip is housed within a support as a single use testing cartridge.

32. The elongated capture strip of any one of Claims 28-31 in which calcium-caging compound is selected from the group consisting of cis-1-(2-bis(carboxymethyl)amino-5-(1-hydroxy-1-(2-nitro-4,5-methylenedioxyphenyl)methyl)phenoxy)-2-(2-bis(carboxymethyl)amino-5-

methylphenoxy)cyclopentane, 1-[2-Amino-5-(1-hydroxy-1-[2-nitro-4,5-methylenedioxyphenyl]methyl)phenoxy]-2-)2'-amino-5'methylphenoxy)ethane-N,N,N',N'-tetraacetic acid, 1-(4,5 dimethoxy-2-nitrophenyl)-1,2 diaminoethane-N, N, N', N'-tetraacetic acid and nitrophenyl-ethylenebis(oxyethylenenitrilo) tetraacetic acid.

33. A plastic cartridge for conducting a binding assay to detect the presence of an analyte in a solution, comprising:
a housing with a receptacle for receipt of a sample, a reservoir containing biotinylated first binding partner immobilized onto paramagnetic particles and a second binding partner conjugated to calcium-sensitive chemiluminescence material, an elongated capture strip within the housing and in fluid communication with the reservoir, said capture strip having a transverse section thereof impregnated with a calcium-caging compound and streptavidin, said transverse section being protected with a light barrier.

34. The plastic cartridge of Claim 32 in which there is a filter between the receptacle and the reservoir.

35. The plastic cartridge of Claim 33 in which there is a filter containing an agent for removal of calcium.

36. The plastic cartridge of any one of Claims 33-35 in which the calcium-sensitive luminescent material is aequorin, Obeln, Mnemiopsin, Berovin, Pholasin, Luciferases or photoproteins isolated from Pelagia, Cypridina and ostracods.

37. Apparatus for carrying out a binding assay comprising a housing enclosing (a) a receptacle to receive the plastic cartridge of any one of Claims 33-36; (b) a means for removing the light protective layer over the transverse stripe; (c) an electromagnet to provide a magnetic field; (e) an ultraviolet light source to project light on a pre-selected portion of the capture strip, and (f) a

photomultiplier disposed to receive light emitted by the pre-selected portion of the capture strip.

38. The apparatus of Claim 37 in which the electromagnet projects multiple magnetic fields along the plastic cartridge.

39. The apparatus of Claim 37 or Claim 38 in which the ultraviolet light source provides light in the range of 250-400 nm.

40. The apparatus of any one of Claims 37-39 in which the photomultiplier detects light in the range of 400-600 nm.

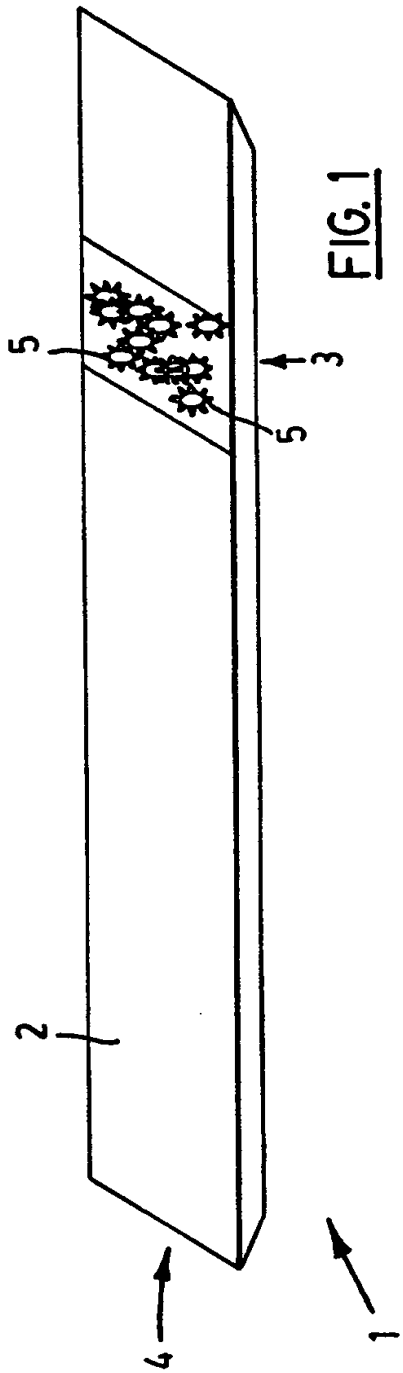


FIG. 1

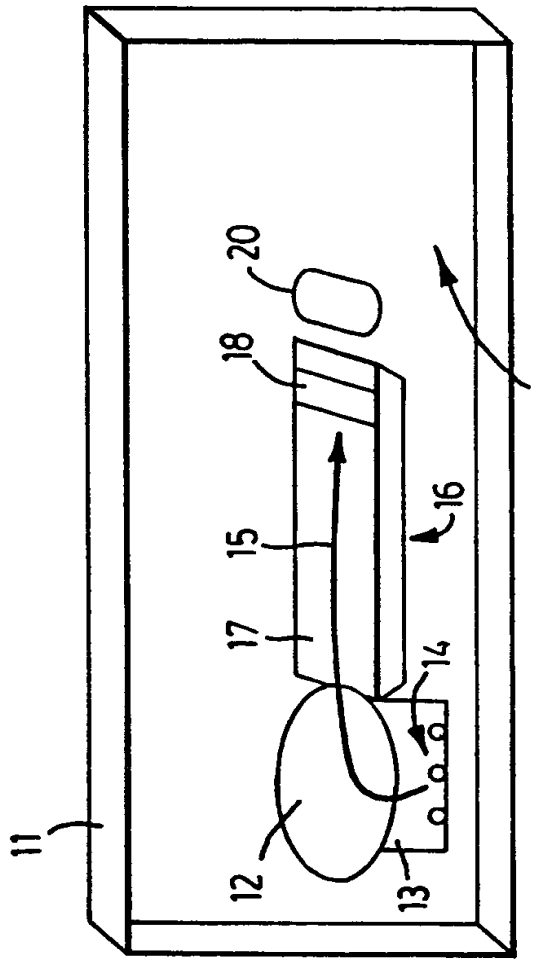


FIG. 2

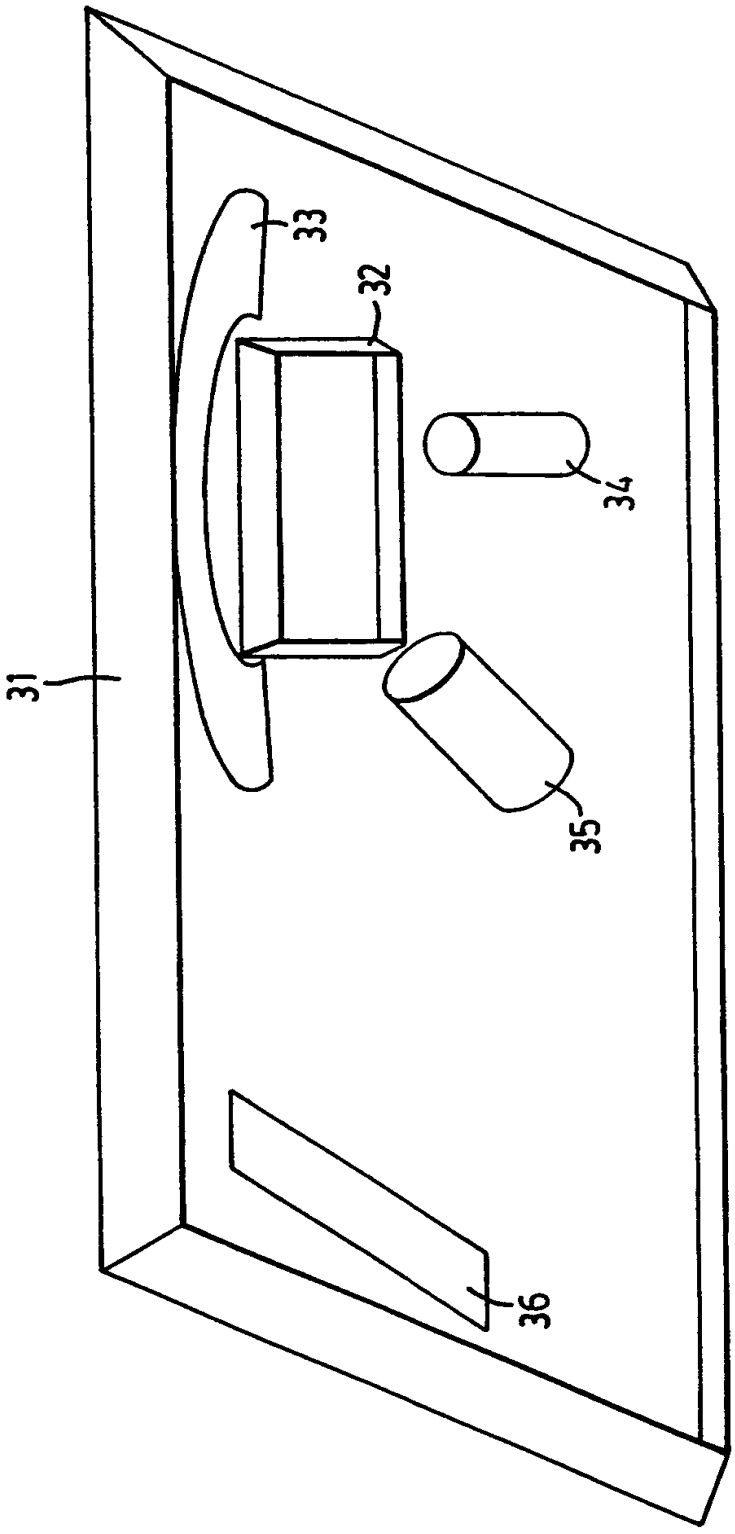


FIG. 3

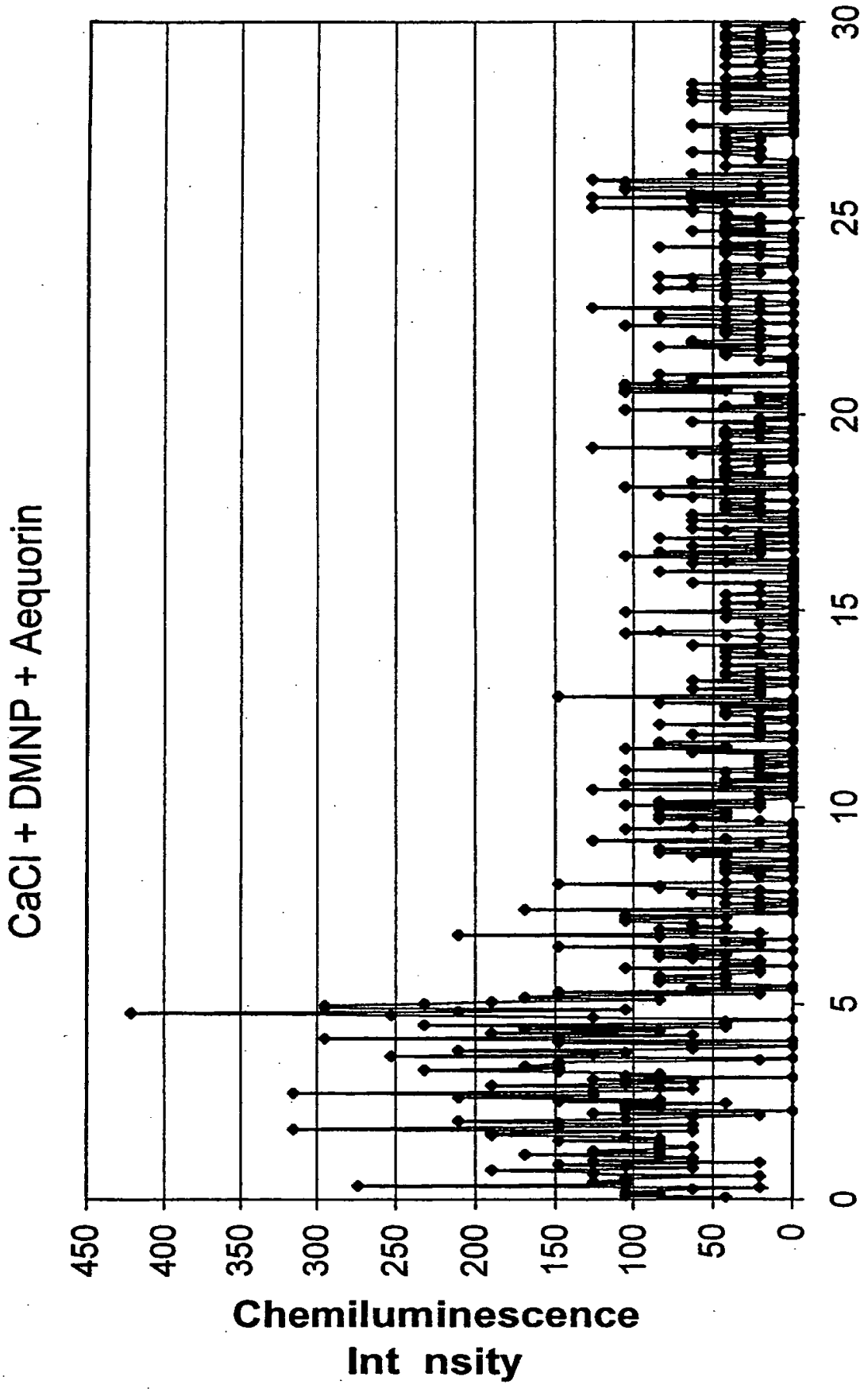

CaCl + DMNP + Aequorin
Chemiluminescence
Int nsity
450
400
350
300
250
200
150
100
50
0
0
5
10
15
20
25
30
Time (s)

FIG. 4

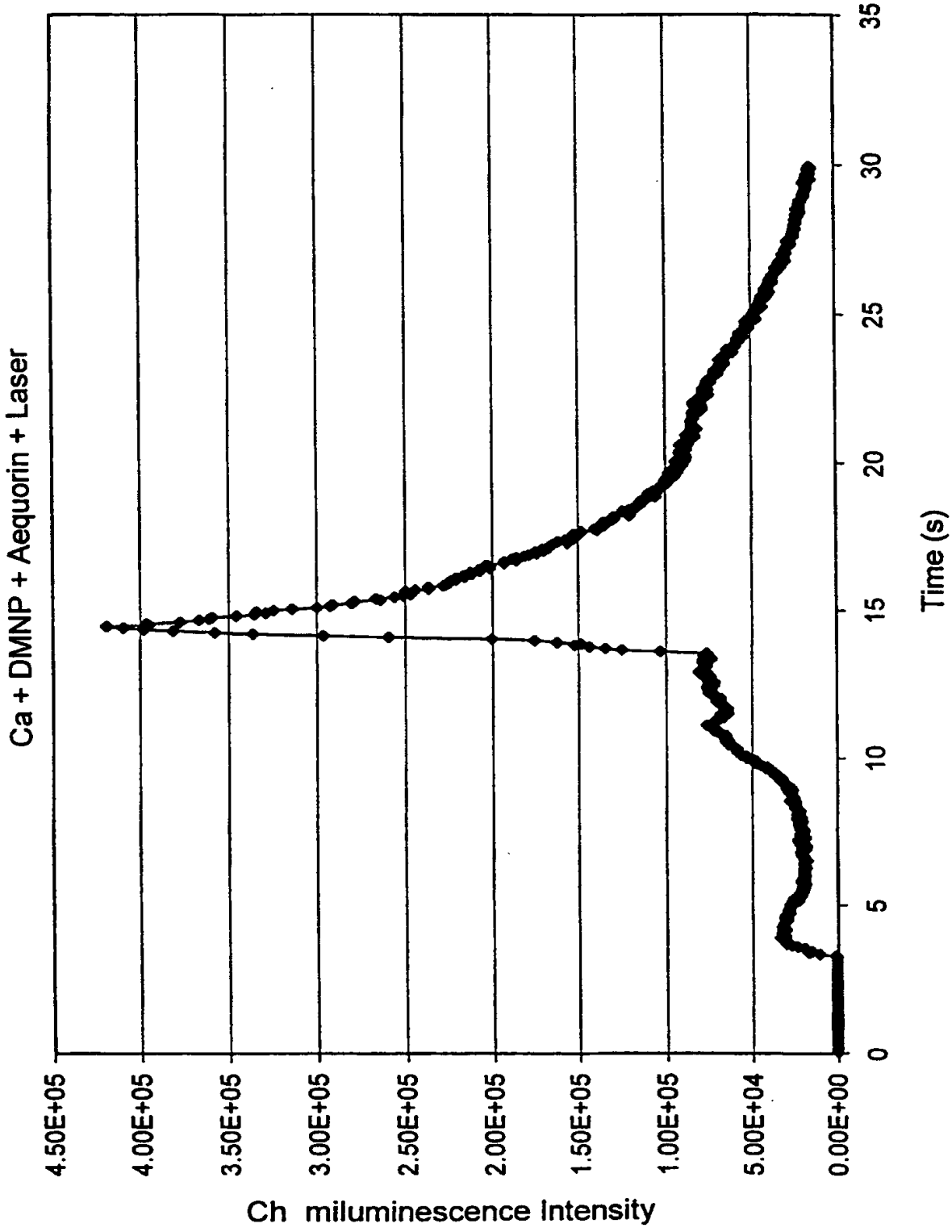

Ca + DMNP + Aequorin + Laser
Ch miluminescence Intensity
4.50E+05
4.00E+05
3.50E+05
3.00E+05
2.50E+05
2.00E+05
1.50E+05
1.00E+05
5.00E+04
0.00E+00
Time (s)
0
5
10
15
20
25
30
35

FIG. 5

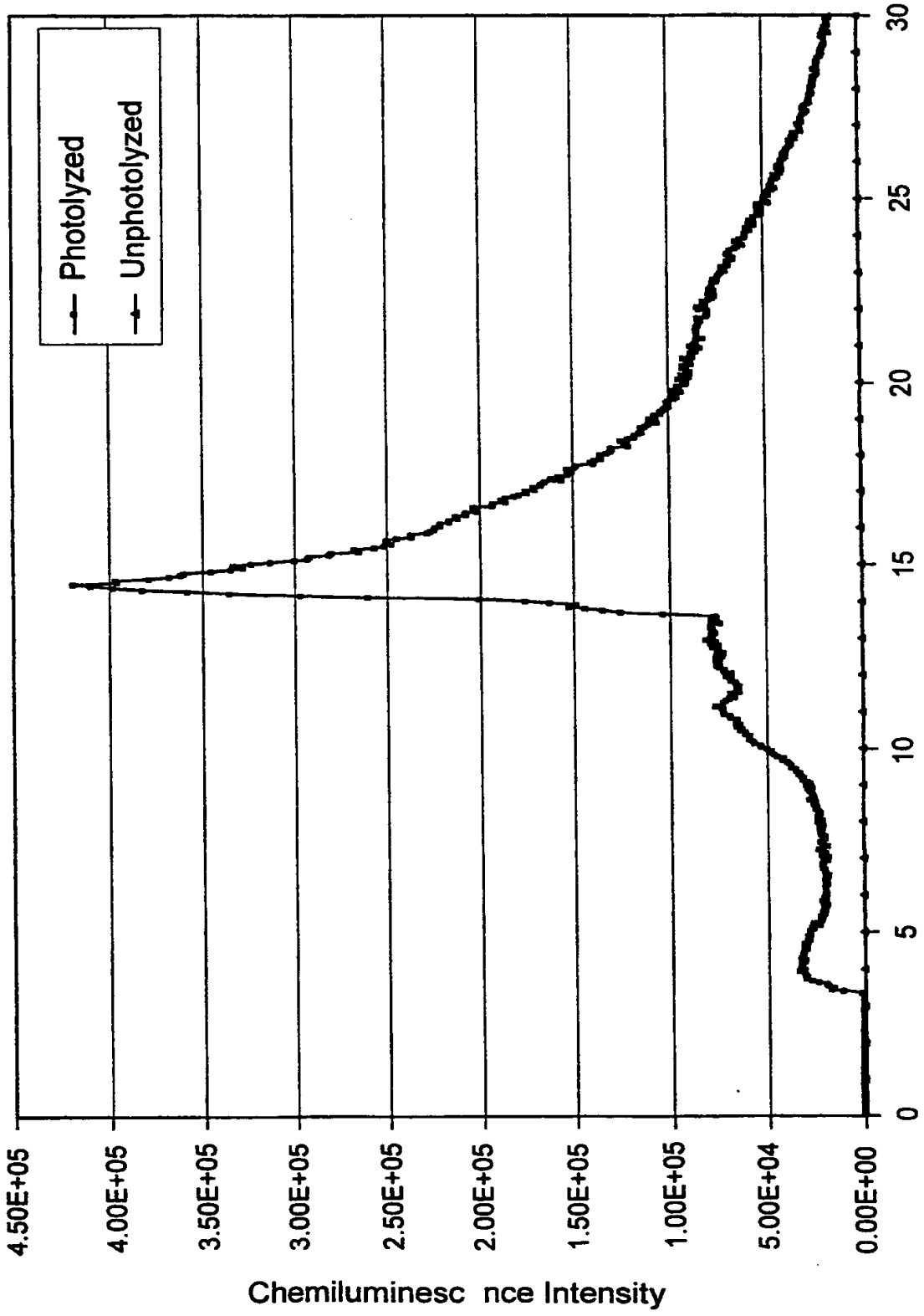

Photolyzed
Unphotolyzed
4.50E+05
4.00E+05
3.50E+05
3.00E+05
2.50E+05
2.00E+05
1.50E+05
1.00E+05
5.00E+04
0.00E+00
Chemiluminesc nce Intensity
0
5
10
15
20
25
30
Time (s)

FIG. 6

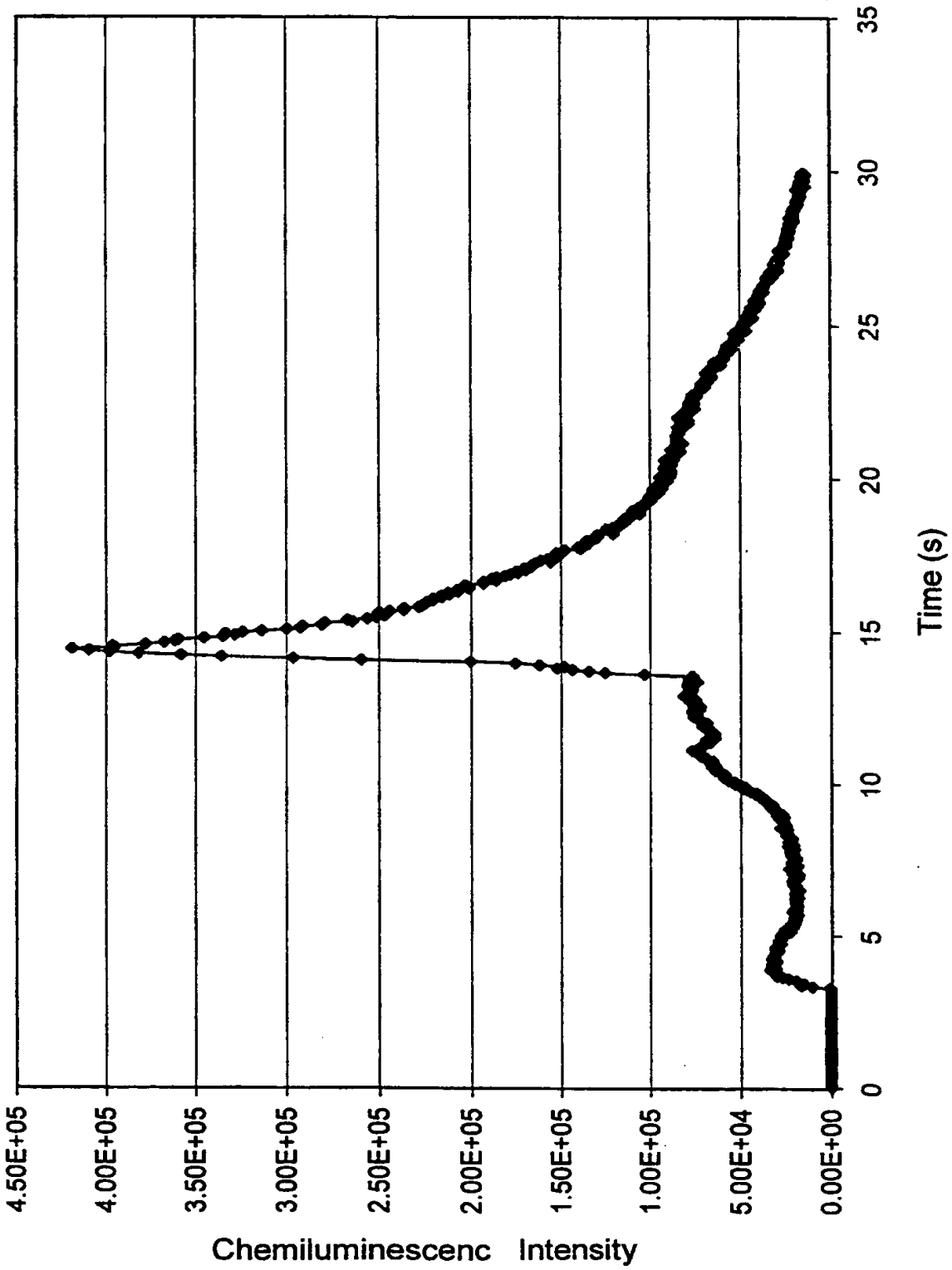

4.50E+05
4.00E+05
3.50E+05
3.00E+05
2.50E+05
2.00E+05
1.50E+05
1.00E+05
5.00E+04
0.00E+00
Chemiluminescenc Intensity
0
5
10
15
20
25
30
35
Time (s)

FIG. 7

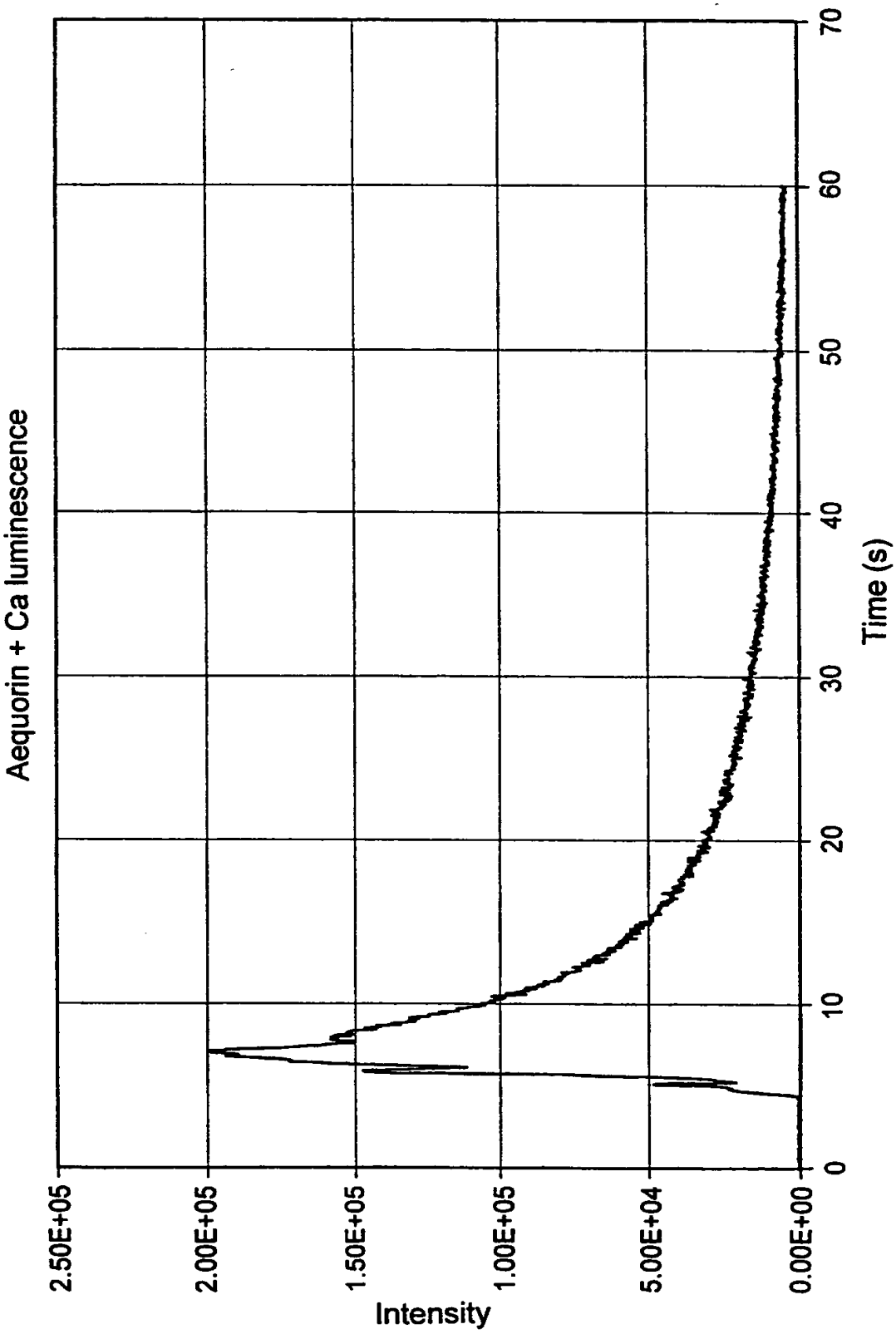

Aequorin + Ca luminescence
Intensity
2.50E+05
2.00E+05
1.50E+05
1.00E+05
5.00E+04
0.00E+00
Time (s)
0
10
20
30
40
50
60
70

FIG. 8